The Journal of Wildlife Management 79(1):122–136; 2015; DOI: 10.1002/jwmg.816

Research Article

Distance to Human Populations Influences Epidemiology of Respiratory Disease in Desert Tortoises

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ABSTRACT We explored variables likely to affect health of Agassiz's desert tortoises (*Gopherus agassizii*) in a 1,183-km^2 study area in the central Mojave Desert of California between 2005 and 2008. We evaluated 1,004 tortoises for prevalence and spatial distribution of 2 pathogens, *Mycoplasma agassizii* and *M. testudineum*, that cause upper respiratory tract disease. We defined tortoises as test-positive if they were positive by culture and/or DNA identification or positive or suspect for specific antibody for either of the two pathogens. We used covariates of habitat (vegetation, elevation, slope, and aspect), tortoise size and sex, distance from another test-positive tortoise, and anthropogenic variables (distances to roads, agricultural areas, playas, urban areas, and centroids of human-populated census blocks). We used both logistic regression models and regression trees to evaluate the 2 species of *Mycoplasma* separately. The prevalence of test-positive tortoises was low: 1.49% (15/1,004) for *M. agassizii* and 2.89% (29/1,004) for *M. testudineum.* The spatial distributions of test-positive tortoises for the 2 *Mycoplasma* species showed little overlap; only 2 tortoises were test-positive for both diseases. However, the spatial distributions did not differ statistically between the 2 species. We consistently found higher prevalence of test-positive tortoises with shorter distances to centroids of human-populated census blocks. The relationship between distance to human-populated census blocks and tortoises that are test-positive for *M. agassizii* and potentially *M. testudineum* may be related to release or escape of captive tortoises because the prevalence of *M. agassizii* in captive tortoises is high. Our findings have application to other species of chelonians where both domestic captive and wild populations exist. Published 2014. This article is a U.S. Government work and is in the public domain in the USA.

KEY WORDS Agassiz's desert tortoise, epidemiology, *Gopherus agassizii*, human census blocks, *Mycoplasma agassizii*, *M. testudineum*, Mojave Desert, period prevalence.

Emerging infectious diseases affect many species of wildlife and, in some cases, pose threats not only to the well-being of specific animal populations but also to biodiversity in general (Daszak et al. 2000, Altizer et al. 2003). Anthropogenic changes to the environment can act as drivers in the emergence of these diseases, e.g., expansion of human populations and encroachment into wild lands, and spillover from captive or domesticated wildlife to wild animal populations (Daszak et al. 2001). Agassiz's desert tortoise (*Gopherus agassizii,* hereafter desert tortoises), part of a species complex of tortoises in the American Southwest (Murphy et al. 2011), provides an example of a species affected by an emerging infectious disease.

In the 1970s, captive desert tortoises with clinical signs of an upper respiratory tract syndrome were common in urban areas of southern California (Saint Amant 1976) and elsewhere. A potential pathogen was not identified during initial studies (Fowler 1976, 1977), but stress and poor nutrition were hypothesized to be predisposing factors (Fowler 1977). Wild desert tortoises with similar clinical signs were first observed in 1988, when tortoises at the Desert Tortoise Research Natural Area in the Mojave Desert, California, were found ill and dying (Jacobson et al. 1991). A population crash followed, and within a few years, the population had declined by 90% (Jacobson et al. 1991, Berry and Medica 1995, Brown et al. 1999). Due in part to the appearance of upper respiratory tract disease (URTD) in wild populations, the desert tortoise was federally listed as a

Received: 18 April 2014; Accepted: 1 October 2014
Published: 9 December 2014

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threatened species and a recovery plan was prepared (U.S. Fish and Wildlife Service, 1990, 1994).

The pathogen causing URTD at the Desert Tortoise Research Natural Area was a new species, *Mycoplasma agassizii* (Brown et al. 1994, 2001). Subsequently, research on mycoplasmal URTD caused by *M. agassizii* focused on several topics: pathogenesis (Jacobson et al. 1995, Homer et al. 1998), transmission from tortoise to tortoise (Brown et al. 1994), clinical signs and subclinical disease (Jacobson et al. 1991, 1995; Schumacher et al. 1997), diagnostic tests (Brown et al. 2002; Wendland et al. 2007, 2010*a*), and chronic disease and mortality (Brown et al. 1999, Christopher et al. 2003). Prevalence was evaluated in relatively small study areas with limited sample sizes in California (Brown et al. 1999, Christopher et al. 2003, Berry et al. 2006), Nevada (Jacobson et al. 1995, Lederle et al. 1997, Schumacher et al. 1997), and Utah (Dickinson et al. 2005). Prevalence and clinical signs of *M. agassizii* also were studied in captive tortoise populations (Johnson et al. 2006, Braun et al. 2014). Substantial related research has been published on the gopher tortoise (*G. polyphemus*) and other species of chelonians (reviewed in Jacobson et al. 2014).

Less is known about a second related pathogen, *M. testudineum,* cultured from desert tortoises with URTD (Brown et al. 2004). Jacobson and Berry (2012) studied pathogenesis and clinical signs in desert tortoises and Brown et al. (2004) conducted limited research on transmission in *G. polyphemus*. A third species of *Mycoplasma*, found on a tortoise in the central Mojave Desert, was identified through DNA fingerprinting (Jacobson et al. 2014).

Species of *Mycoplasma* are well known as etiologic agents of respiratory diseases in many hosts, including many farm, laboratory, companion animals, and humans; thus, a considerable body of information has accumulated about them (Simecka et al. 1992, Brown et al. 2002). In generalizing about hosts, Brown et al. (2002) stated that hosts do not typically clear mycoplasmal infections and once a host is infected, it "… is presumed to be infected for life."

Epidemiology and risk factors for *Mycoplasma* spp. on a landscape scale have yet to be explored in depth for desert tortoises. In 2004, the Department of the Army received approval from the USFWS to translocate several hundred desert tortoises from the National Training Center (NTC) at Fort (Ft.) Irwin in the central Mojave Desert (USFWS 2004, Esque et al. 2005). The study area covered about 1,180 km^2. As part of the translocation project, we evaluated the tortoises to be translocated and resident tortoises that might be affected by the project for health, specifically for the 2 pathogens known to cause mycoplasmal URTD in desert tortoises. This large-scale translocation project at a landscape level provided a unique opportunity to identify potential risk factors that might contribute to prevalence of mycoplasmal disease in wild desert tortoise populations. Identification of potential risk factors for URTD is a necessary step in future efforts to reduce or control the spread of disease in wild tortoise populations.

With the large scale of the project, we addressed several questions associated with epidemiology prior to translocation: 1) how were tortoises with positive tests for *M. agassizii* and *M. testudineum* distributed in comparison to tortoises with negative tests? and 2) does probability of infection as measured by exposure to *M. agassizii* and *M. testudineum* vary with sex and carapace length of tortoises, physical and biotic variables (i.e., slope, aspect, elevation, and vegetation), proximity to a tortoise with positive tests for *M. agassizii* or *M. testudineum*, or anthropogenic variables? The anthropogenic variables of interest were distances to roads, areas devoid of vegetative habitat for tortoises (i.e., agricultural areas, dry lake beds, playas), urban areas, human census blocks, and human population density. Our questions represent multiple hypotheses we developed to explain mechanisms by which different variables on the individual or landscape level could increase risk to mycoplasmal exposure (Table 1). Our ultimate objective was to develop models for predicting where tortoises with positive tests for the 2 pathogens were most likely to occur.

STUDY AREA

The study area encompassed an estimated 1,183 km^2 in the central Mojave Desert, California, northeast of the city of Barstow (population 22,836; 2010 Census) and north of the unincorporated communities of Yermo (population estimate 1,500–2,000), the Marine Corps Logistics Base, Nebo (population estimate 1,724; 2000 Census), and Daggett (population estimate 1,000–2,500) in San Bernardino County, California (U.S. Census Bureau 2000, 2010 Fig. 1). The population in the Ft. Irwin cantonment north of the study area was 9,581 (U.S. Census Bureau 2000). Interstate freeway I-15 formed the southern boundary and the Calico Mountains the western and southwestern boundaries. The northern boundary was within the Army's NTC at Ft. Irwin and also in designated critical habitat for the tortoise (USFWS 1994). The eastern boundary was the Soda Mountains.

The composition of perennial vegetation was primarily shrubs in alliances associated with creosote bush (*Larrea tridentata*) and white bursage (*Ambrosia dumosa*), cheesebush (*Ambrosia hymenoclea*), and California joint fir scrub (*Ephedra californica*; California Department of Fish and Game 2010). Allscale scrub (*Atriplex polycarpa*, *A.* spp.) alliances bordered Coyote Lake (playa) and edges of other smaller playas. Elevations ranged from 540 m at Coyote Lake in the central part of the study area to 1,280 m in the Calico Mountains. Annual precipitation, measured 7.7 km south of the southern edge of the study area at the Barstow Daggett Airport, California (34°51′ N, –116°47′ W, 584 m), averaged 105.92 mm, of which approximately 60% occurred primarily between 1 October and 31 March (National Oceanic and Atmospheric Administration, National Climate Data Center 2004–2008).

METHODS

Collecting Data From Desert Tortoises

Sampling of desert tortoises was uneven across the study area and based on three factors: objectives, terrain, and land

Table 1. A summary of hypotheses about predictor variables with expected outcomes for epidemiological models of *Mycoplasma agassizii* and *M. testudineum* in Agassiz's desert tortoises in the central Mojave Desert, California.

Type and definition of variable	Hypothesis: direction of effect – features having higher prevalence of infected tortoises	References
Anthropogenic variables		
Distance to nearest centroid of a human-population census block	Negative—shorter distance to the center of a human population census block, where infected tortoises may escape or be released	Berry et al. (2006), Johnson et al. (2006)
Distance to nearest urban area	Negative—shorter distance to an urban area, where infected tortoises may escape or be released	Berry et al. (2006), Johnson et al. (2006), Jacobson et al. (1995) Schumacher et al. (1997)
Human population density for each census block	Positive—higher human population density	Same as above
Housing density at nearest centroid of a census block	Positive—higher housing density	Same as above
Distance from areas devoid of vegetation (agricultural fields, playas)	Negative—shorter distance to old agricultural fields and playas	Same as above; also Chaffee and Berry (2006), Kim et al. (2014) and potential stressors from agricultural use
Distance from interstate freeway, I-15	Negative—shorter distance to interstate freeway, I-15, where infected tortoises may be released	Berry et al. (2006)
Distance from secondary roads	Negative—shorter distance to secondary roads	
Distance from tertiary roads (all other roads)	Negative—shorter distance to tertiary roads	
Habitat variables		
Elevation	Negative—lower elevations, where roads and housing are more common	
Vegetation type	Variable—vegetation types having higher densities of tortoises	
Slope	Negative—lower slopes (but not lowest parts of valleys) where tortoise densities are likely to be higher	
Aspect	Variable—aspects having higher densities of tortoises, e.g., north-facing slopes are more mesic and may have more shrub cover and forage	
Disease variables		
Distance from other tortoises test-positive for *M. agassizii* or *M. testudineum*	Negative—shorter distance to other infected tortoises that can transmit disease via direct contact	Brown et al. (1994); Ruby and Niblick (1994)
Tortoise variables		
Sex	Variable—sex differences, due to health attributes (hematology, plasma biochemistry) that might influence susceptibility to infection	Christopher et al. (1999)
Carapace length at the midline	Positive—larger size, due to dominant tortoises potentially having more social interactions and more exposure	Niblick et al. (1994)

ownership. Within the southern boundaries of Ft. Irwin, all tortoises scheduled to be translocated required evaluation. Outside Ft. Irwin, the objective was to sample resident tortoises in areas where the translocatees were likely to be placed. Because few tortoises occurred in rough, steep terrain in the Paradise, Calico, Alvord, and Soda mountains and on or adjacent to Coyote Lake, sampling was sparse in these areas (Fig. 1). We did not sample tortoises on private lands.

Evaluating tortoises for mycoplasmal URTD presented challenges at several levels: field observations of clinical signs, field sampling and quality control, existing laboratory tests, and the limitations of information available from each test. We evaluated desert tortoises for clinical signs of health and disease and collected blood and nasal flush samples for diagnostic tests during 2 periods: 1) spring and 2) late summer and early fall, when they were more likely to be active and above ground (Nagy and Medica 1986, Zimmerman et al. 1994). Evaluations occurred during spring 2005 (31 May–8 Jun), summer and fall 2005 (22 Sep–1 Oct), summer and fall 2006 (19 Sep–20 Oct), spring 2007 (15 May–8 Jun), summer and fall 2007 (23 Sep–7 Nov), spring 2008 (23 Apr–6 Jun), and summer and fall 2008 (23 Sep–26 Oct). We evaluated most tortoises between 2005 and 2007, prior to the spring 2008 translocation; we also examined a small group of resident tortoises in 2008. We recorded clinical signs of health and disease using a form modified from Berry and Christopher (2001) and digital photographs of various body parts, in particular the beak, nares, and eyes. We emphasized clinical signs of URTD caused by *M. agassizii, M. testudineum*, and other potentially infectious diseases, e.g., serous, mucoid, or purulent nasal discharge; occluded or partially occluded nares; excessive tearing to purulent ocular drainage; conjunctivitis; and moderate to severe edema of the palpebrae and periocular area (Jacobson et al. 1991, Brown et al. 1994, Schumacher et al. 1997, Jacobson and Berry 2012). Although we recorded ocular signs, we did not use them in the clinical analysis because ocular signs are often observed in diseases other than mycoplasmosis, change as the tortoise is handled, and vary with foraging status (Homer et al. 1998, Christopher et al.

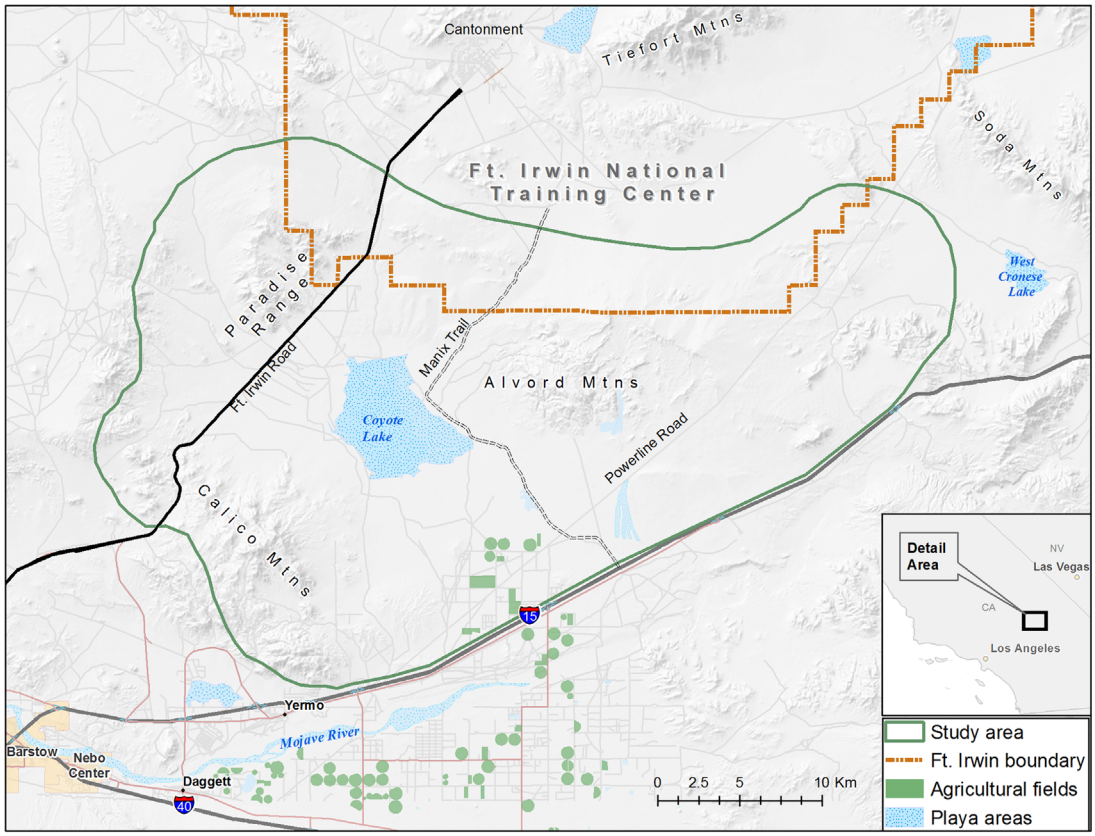

Figure 1. Diagram of the study area for Agassiz's desert tortoise in the central Mojave Desert, California, in 2004 with place names used in the analysis of epidemiological data. The inset map shows the general location of the study area in the American Southwest.

2003, Boyer 2006; K. H. Berry, U.S. Geological Survey, personal communication). We recognized that clinical signs typical of mycoplasmosis (e.g., purulent nasal discharge) alone were not a reliable means of determining whether tortoises have mycoplasmosis, because significant numbers of tortoises may be subclinical, that is have no clinical signs but positive serology (34%; Schumacher et al. 1997, Jacobson and Berry 2012) and lesions consistent with URTD in the upper respiratory tract (Jacobson et al. 1995). Another drawback of relying solely on clinical signs is that clinical signs may develop from 2 to 8 weeks after exposure (Brown 2002).

We used 3 laboratory tests for *M. agassizii* and *M. testudineum*: an enzyme-linked immunosorbent assay (ELISA) that can detect antibodies to *M. agassizii* (Wendland et al. 2007, 2010*a*), a second ELISA that that can detect antibodies to *M. testudineum* (a modification of the ELISA for *M. agassizii*), and direct culture of nasal lavages combined with polymerase chain reaction (PCR) and DNA identification (Brown et al. 2002, 2004; Jacobson et al. 2014). The ELISA indicates whether a tortoise has been exposed to the pathogens and has responded by producing anti-*Mycoplasma* antibodies. We used a refined ELISA for *M. agassizii* that has a sensitivity of 0.98, specificity of 0.99, and a Youden index, J, of 0.98 (Wendland et al. 2007). The positive and negative predictive values (PPV, NPV) are a function of seroprevalence, e.g., the PPV drops below 90% only at a seroprevalence of <9% and the NPV drops below 90% at a >85% seroprevalence (Wendland et al. 2007). Of the clinical and diagnostic tests available, the ELISA has most closely reflected whether lesions typical of mycoplasmosis are present in the upper respiratory tract (Jacobson et al. 1995, Jacobson and Berry 2012).

For the ELISAs, we drew samples of blood (approx. 0.5 – 1.0 cc) from tortoises either by brachial venipuncture or from the subcarapacial site using standard protocols (Hernandez-Divers et al. 2002). We considered samples of blood containing 15% or more of lymph suboptimal because of the potential for false negative test results with dilution (e.g., Gottdenker and Jacobson 1995). Where possible, we repeated such samples to obtain 95–100% blood with no lymph or only a trace of lymph. The ELISAs also may give false negative results if the tortoise is exposed to one or both pathogens a few weeks prior to blood sampling and insufficient time has elapsed for mounting an antibody response.

We took a nasal lavage for culturing potential species of *Mycoplasma* in the nasal cavity (Brown et al. 2002, Berish et al. 2010) and flushed the nares with 10 ml of sterile phosphate-buffered 0.9% saline using a 10-cc syringe with a 0.22-gauge catheter attached (stylus removed). Each naris received approximately 5 ml. We collected the lavage in a sterile specimen cup and then added SP4 medium (1 ml) to stabilize and enhance viability of mycoplasmas during transport. Some tortoises swallowed a portion or all of the

flush, necessitating an additional flush, and potentially diluting the lavage. Cultures have the advantage of providing direct proof of infection at the time the tortoise was sampled; however, the proportion of false negative or uninformative samples is high because mycoplasmas are fastidious, take weeks to grow, and the culture is subject to microbial contamination (Brown et al. 2002).

We chilled blood and nasal samples in the field, centrifuged blood samples, and separated plasma within 3 hours. We then placed both blood and nasal samples on dry ice and froze them at −80°C. We submitted 9 tortoises seropositive for *M. testudineum* for necropsy as part of a related project on pathogenesis of *M. testudineum* (Jacobson and Berry 2012).

Federal and State endangered species permits to conduct this research were to W. Quillman and C. Rognan at the NTC, Ft. Irwin (U.S. Fish and Wildlife Service Recovery Permit TE-102235-1, -2, and -3, and California Department of Fish and Game Memorandum of Understanding [MOU] 801179-01). Similar permits, including salvage and necropsy, were held by K. H. Berry (U.S. Fish and Wildlife Service, TE-06556-12 and -13; California Dept. of Fish and Game MOU 801063-04 and Scientific Collecting Permit 003623).

Data Analysis

We used 3 criteria to include a tortoise record in the analyses: 1) the tortoise was a resident tortoise in its home range (had not been moved or translocated); 2) we evaluated the health of the tortoise at the time we collected tissue samples; 3) if we evaluated the tortoise multiple times, then we used the most recent evaluation prior to spring of 2008 (time of translocation), unless the tortoise had a suspect or positive test for *Mycoplasma* spp. between 2005 and 2007, in which case we used that record instead. One thousand four large immature and adult tortoises met the criteria.

Diagnostic testing.—The Mycoplasma Research Laboratory at the University of Florida performed all diagnostic tests for *M. agassizii* and *M. testudineum*. Descriptions of the serological tests and culture and PCR techniques on nasal flush samples are in Brown et al. (2001) and Wendland et al. (2007, 2010*a*). For cultures, the lab team inoculated a 0.4-ml aliquot of the nasal flush sample into 4 ml of SP4 broth. They removed 2 ml of the inoculated broth and filtered it using a 0.45-μm filter to remove potential fungal contaminants. They incubated both filtered and unfiltered broth cultures at 30°C and observed for color change. They plated 20 μl of each broth sample and the nasal lavage fluid on SP4 agar, then they analyzed broth cultures for the presence of *M. agassizii* DNA based on PCR amplification of a portion of the 16S rRNA gene (Brown et al. 1995). Using AgeI or Nci I on all positive PCR samples, they conducted restriction fragment length polymorphism analysis of the 16 S rRNA gene to confirm that the isolates were either *M. agassizii* or *M. testudineum* (Brown et al. 1995, Berish et al. 2010, Wendland et al. 2010*a*).

Spatial data layers.—We acquired several geographic information system (GIS) layers from the United States Census Bureau (http://www.census.gov/geo/www/tiger/, accessed 26 Nov 2008) including: urban area polygons; census blocks; and data tables containing population, number of housing units, and number of households for each census block as of 2004. Twenty-three census blocks with at least 1 person each were within the study area; however, large areas had no people or households and therefore no census blocks (Fig. 2; see Supplemental Information for a listing of census blocks, human population, number of households, and household units in the study area). We created a single point in the center of each census block as the centroid using the GIS Feature to Point tool that creates points (Environmental Systems Research Institute, Inc. [ESRI] 2009*a*; Fig. 2). We calculated population density for each census block by taking the population number and dividing by the area in km^2. We acquired a general vegetation layer from the United States Bureau of Land Management (BLM; http://www.blm.gov/ca/gis/, accessed 28 Oct 2010). We calculated elevation at each tortoise location using a raster value extraction tool (ESRI 2009*b*) based on a 30-m digital elevation model (DEM) acquired from the National Elevation Dataset (NED; http://seamless.usgs.gov/, accessed 21 Sep 2010). We created slope and aspect surfaces based on the DEM layer using an inverse distance-weighted surface interpolation method (ESRI 2009*c*). We compiled the road layer by merging TIGER road layers (www.census.gov/geo/www/tiger/, accessed 28 Oct 2010), BLM road layers (http://www.blm.gov/ca/gis/, accessed 28 Oct 2010), and any additional roads using global positioning system (GPS) track logs. Prior to analysis, we assigned the roads in this layer to 1 of 3 categories (primary, secondary, and tertiary) depending on amounts of vehicle traffic. The single primary road was the interstate, I-15; secondary roads included the paved Ft. Irwin Road, Manix Trail (non-paved), and a power line road (non-paved); and tertiary roads were defined as all other non-paved roads. We created a GIS layer for dry lake beds (playas) and old agricultural fields using aerial photographs (2005) from the National Agriculture Imagery Program. These 2 types of areas were either naturally bare of vegetation (playa, dry lake bed) or had been cleared of native vegetation for agriculture and thus were unusable by tortoises. We used the phrase "devoid of vegetation" to describe them.

We imported a single location for each desert tortoise, specifically the site where we took blood and nasal samples, into a GIS layer and recorded the covariates identified below for each location. Using the Near Analysis tool (ESRI 2009*d*), we calculated distances (m) of each tortoise to primary, secondary, and tertiary roads; edge of the nearest urban area polygon; edge of the nearest polygon devoid of vegetation; the centroid for the nearest human-populated census block (Fig. 2), and other tortoises with antibody positive or suspect ELISA tests or positive cultures.

Statistical analyses.—We defined a tortoise as test-positive or infected for 1 of the 2 species of *Mycoplasma* if it was positive by culture and PCR or positive or suspect for specific antibody by an ELISA test for that *Mycoplasma* species. We defined tortoises as test-negative if they were antibody negative for *M. agassizii* or *M. testudineum* with the ELISA tests and were also culture and PCR negative for the

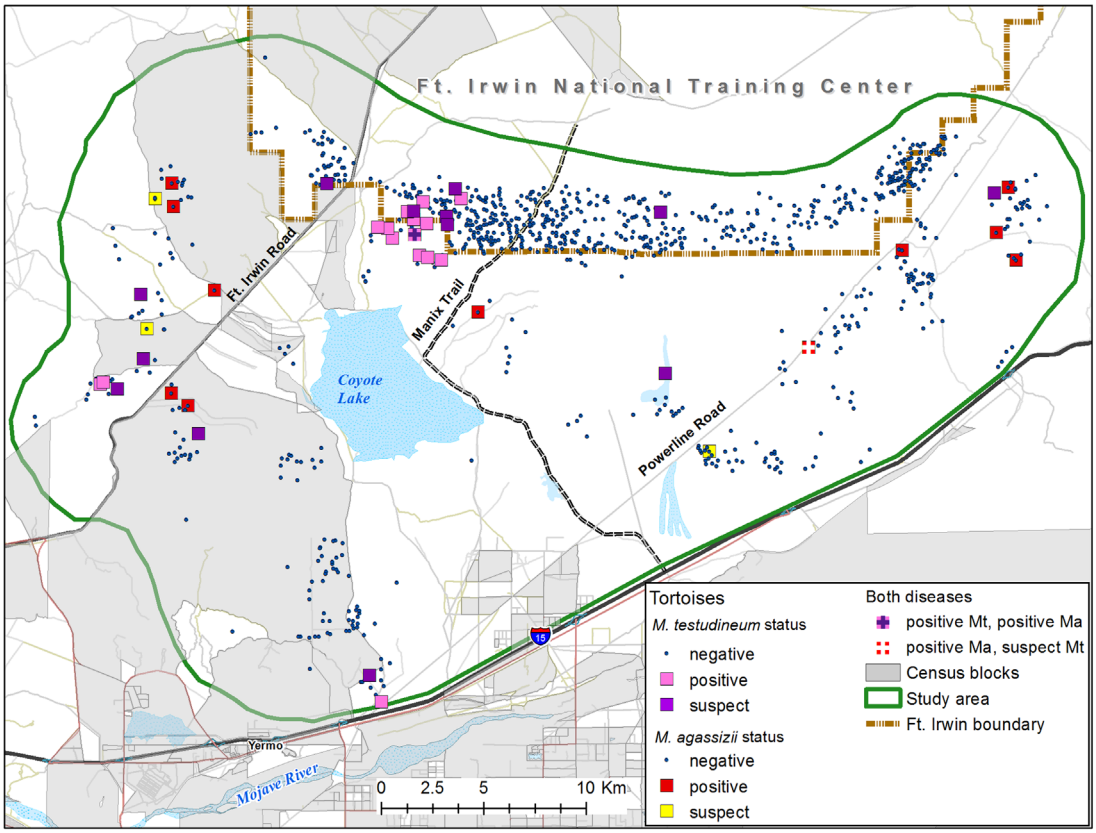

Figure 2. Distribution of Agassiz's desert tortoises with positive or suspect enzyme-linked immunosorbent assay (ELISA) tests or positive cultures for *Mycoplasma agassizii* and *M. testudineum* or both species of *Mycoplasma* within or outside of human census blocks of different house densities at the study area in the central Mojave Desert, California in 2005–2008. The census blocks with people are shaded grey, whereas areas with no people and no census blocks are white. Outlines of each of 23 census blocks and their centroids used in the epidemiological models are in Supplemental Information. In the legend, Ma = *Mycoplasma agassizii* and Mt = *M. testudineum*.

Mycoplasma species of interest. We defined period prevalence as the proportion of tortoises with a test-positive result at any time during the 4-year period from 2005 through 2008 (hereafter prevalence). We analyzed prevalence for *M. agassizii* and *M. testudineum* separately, except when indicated. We conducted all statistical analyses using R software (R Version 3.0.2, www.r-project.org, accessed 22 Jan 2014). We used Syrjala's modification of the Cramér-von Mises test to compare differences in the spatial distributions of test-positive outcomes between the 2 *Mycoplasma* species (R package ecespa; Syrjala 1996, De la Cruz 2008). We also analyzed the independence between the 2 *Mycoplasma* species by conducting Fisher's exact test to determine whether tortoises that were test-positive for one disease were more or less likely to be test-positive for the other disease (R Development Core Team 2012).

Our hypotheses involved covariates based on 1) the physical and biological environment—elevation, aspect, slope, and vegetation type; 2) individual characteristics of tortoises—sex and midline carapace length (MCL); 3) disease exposure—distance from other test-positive tortoises; and 4) anthropogenic influences (Table 1). For anthropogenic influences, we analyzed covariates based on distances to the nearest centroid of a human-populated census block (shortened to human population centroid); edge of urban area; areas devoid of vegetation; and primary, secondary, and tertiary roads; as well as human population density and housing unit density at the nearest human population centroid. In general, we hypothesized that the closer to human presence, the more likely that tortoises are infected because humans often have captive tortoises and a high percentage of these are infected with *M. agassizii*. We assessed multicollinearity by calculating variance inflation factors (VIF) among continuous covariates and generalized VIF (GVIF) when including the categorical factor of vegetation type (Fox and Weisberg 2011). We avoided models that considered all effects simultaneously because multicollinearity among these covariates was high (maximum GVIF = 15.6, maximum correlation [r] = 0.83).

We mitigated for multicollinearity by eliminating covariates based on comparisons of single-covariate logistic regression models for *Mycoplasma* status (1 = test-positive, 0 = test-negative; McCullagh and Nelder 1989, Agresti 2002, R Development Core Team 2012). We compared each model to the null model without any covariate. We used second-order corrected Akaike's Information Criterion (AIC_c) to identify and retain covariates with the strongest evidence for predicting disease, defined by models with AIC_c less than 2 units below that of the null model and >0.01 Akaike weight (Burnham and Anderson 2002). We examined the signs (positive or negative) of model

coefficients to assess consistency with the relationships we hypothesized (Table 1).

With the covariates retained by AIC, we used regression trees to develop a predictive model based on multiple predictors by recursively separating the tortoises into groups that differed significantly in prevalence, starting by partitioning the covariate attributed with the most significant effect. This approach flexibly explored combinations of covariate classes and resulted in a tree-structured partition of tortoises with different prevalences (De'ath and Fabricius 2000). We used significance testing at a type 1 error of 0.05 as the partitioning criterion (R package party; Hothorn et al. 2006). By filtering covariates prior to constructing regression trees, we may have missed opportunities to discover interaction effects among the eliminated covariates. However we believe this risk was minimal because we eliminated only those covariates that demonstrated no evidence of relationship with infections in one-on-one comparisons.

RESULTS

Of the 1,004 desert tortoises evaluated, 389 were females and 615 were males. With the exceptions of 2 large immature females and 1 large immature male, all tortoises were small to large adults $\geq$180 mm MCL. For the entire sample, sizes of tortoises ranged from 174 mm to 279 mm MCL for females and from 179 mm to 313 mm MCL for males. The prevalence of tortoises with antibody-positive or suspect ELISA tests and cultures was low: 1.49% (15/1,004) for *M. agassizii* and 2.89% (29/1,004) for *M. testudineum.* Four tortoises were antibody positive or suspect for 2 tests or cultures: 1 tortoise was antibody positive for both species of *Mycoplasma,* 1 tortoise was antibody positive for *M. agassizii* and antibody suspect for *M. testudineum*, 1 tortoise was antibody positive and culture positive for *M. agassizii*, and 1 tortoise was antibody suspect and culture positive for *M. testudineum* (Table 2).

We did not observe clinical signs for mycoplasmal URTD, specifically serous nasal discharge or damp or wet naris or nares predictive of antibody positive ELISA tests, in any of the 15 test-positive tortoises for *M. agassizii* (Table 2). In contrast, we observed 13.8% (4/29) of tortoises test-positive for *M. testudineum* with signs of nasal discharge (Table 2). Potential signs of a recent nasal discharge (partially occluded or occluded naris or nares or dried exudate on beak and or forelimbs), were evident in 46.7% (7/15) of test-positive tortoises for *M. agassizii* and 31.0% (9/29) for *M. testudineum*. The remaining tortoises that were test-positive for *M. agassizii* (53.3%, 8/15) and *M. testudineum* (55.2%, 16/29) had no clinical signs associated with the beak or nares.

Visual inspection of the distributions of tortoises test-positive for *M. agassizii* and *M. testudineum* indicated some apparent differences. Whereas tortoises with *M. agassizii* occurred throughout the study area, tortoises with *M. testudineum* occurred primarily in the west half of the study area, west of the Manix Trail (Fig. 2). Only 2 tortoises were test-positive for both species of *Mycoplasma*, indicating that the pathogens in our test population rarely co-occurred and may be acting independently (Fisher's Exact test, $P = 0.067$). However, the spatial distributions of test-positive tortoises did not differ statistically between *M. agassizii* and *M. testudineum* (Syrjala's test, $P = 0.1514$, 9,999 permutations).

Single Variable Models and Regression Tree for *M. agassizii*

The best predictor of *M. agassizii* status in desert tortoises was distance to the nearest centroid of a human-populated census block (Fig. 2). This predictor had the majority of the Akaike weight (0.88; Table 3). The distance to a human population centroid was negatively associated with prevalence of test-positive tortoises (Table 3, Fig. 3). Other predictors included distances to urban areas, tertiary roads, and other test-positive tortoises (Table 3). Positive or suspect disease status was negatively associated with distance to other test-positive tortoises and distance to tertiary roads and positively associated with distance to an urban area, suggesting that greater prevalence of disease occurred with shorter distances to other test-positive tortoises and to unpaved, tertiary roads but longer distances from urban areas

Table 2. Clinical signs (related to nasal discharge) of mycoplasmal upper respiratory tract disease (URTD) in Agassiz's desert tortoises with positive or suspect enzyme-linked immunosorbent assay (ELISA) tests and cultures in the central Mojave Desert, California, between 2005 and 2008. Four tortoises were suspect or positive for 2 tests (see superscripts).

	Clinical signs (nasal) of mycoplasmal URTD			No clinical signs of nasal discharge, occluded nares, or dried exudate
Laboratory test results	Nasal discharge; moist/damp/ wet nares	Partially occluded or occluded naris or nares	Dried exudate on beak and or forelimbs	
M. agassizii				
ELISA +		3[a,b]	1	6[c]
ELISA suspect		2		1
Culture +		1[b]	1	1
M. testudineum				
ELISA +	2	3	1	8[c]
ELISA suspect	2	3[a]	2	8[d]
Culture +				1[d]

[a] 1 tortoise was ELISA+ for *M. agassizii* and ELISA suspect for *M. testudineum.*
[b] 1 tortoise was ELISA+ and culture+ for *M. agassizii.*
[c] 1 tortoise was ELISA+ for both *M. agassizii* and *M. testudineum.*
[d] 1 tortoise was ELISA suspect and culture+ for *M. testudineum.*

Table 3. Covariates associated with the probabilities for test-positive disease status for *Mycoplasma agassizii* and *M. testudineum* among Agassiz's desert tortoises in the central Mojave Desert, California, between 2005 and 2008, based on logistic regression models. All models contain 1 covariate, except for the Null model which assumes a constant rate. All models are ranked by second order corrected Akaike's Information Criterion (AIC_c) and listed from best to worst with the number of model parameters (K), –2 log likelihood (–2LL), difference in AIC_c relative to the best model (ΔAIC_c), Akaike weight (ωAIC), and covariate parameter ± standard error (SE). The sign of the covariate parameter indicates the direction of effect. Results are shown only for the null model and models with AIC_c less than 2 units below that of the null model and >0.01 Akaike weight. Not applicable = n/a.

Model	K	–2LL	AIC_c	ΔAIC_c	ωAIC	Parameter ± SE
Mycoplasma agassizii						
DHPOPC[a]	2	143.06	147.07	0	0.87	–0.28 ± 0.088
DURBAN[b]	2	149.60	153.61	6.54	0.03	0.12 ± 0.049
DTER[c]	2	149.96	153.97	6.90	0.03	–0.74 ± 0.369
DPOS[d]	2	150.20	154.21	7.13	0.02	–0.26 ± 0.124
Null	1	155.89	157.89	10.82	<0.01	n/a
Mycoplasma testudineum						
DHPOPC	2	233.72	237.73	0	0.98	–0.32 ± 0.067
DVOID[e]	2	241.24	245.26	7.52	0.02	–0.28 ± 0.069
Null	1	262.73	264.74	27.00	<0.01	n/a

[a] DHPOPC = distance to nearest centroid of a human-populated census block.
[b] DPOS = distance from other tortoises test-positive for *M. agassizii*.
[c] DTER = distance from tertiary roads (all other roads).
[d] DURBAN = distance to nearest urban area.
[e] DVOID = distance from areas devoid of vegetation.

(Fig. 3). The 2 urban areas were the city of Barstow and cantonment at Ft. Irwin (Fig. 1).

We detected little multicollinearity among these 4 distance covariates: human population centroid, urban areas, tertiary roads, and other test-positive tortoises (max. VIF = 1.1, $r = 0.56$); a regression tree with these covariates resulted in 5 classes (Fig. 4). The first predictor, distance to a human population centroid, was partitioned because tortoises test-positive for *M. agassizii* comprised 18% (2/11) of tortoise locations at short distances (≤1.2 km) from human population centroids compared with only 3.5% (5/141) at medium distances (1.2–3.9 km) and the lowest prevalence of 0.94% (8/852) at greater distances (>3.9 km). Most of the 8 test-positive tortoises in the latter category occurred ≥24.3 km from urban areas for a prevalence of 3.9% (5/129) compared to 0.41% (3/723) at shorter distances to urban areas. Among tortoises located farthest from a human-population centroid (>3.9 km) and ≥24.3 km from urban areas, the regression tree identified further variations in *M. agassizii* prevalence associated with distance to other test-positive tortoises. Contrary to our hypothesis and to the pattern indicated by the logistic regression (Table 3), the regression tree indicated that tortoises located ≥4.0 km from test-positive tortoises had a 33% (2/6) prevalence for *M. agassizii* compared with 2.4% (3/123) of tortoises located closer. The regression tree did not partition tortoises based on distance to tertiary roads, although it was important when used alone in the logistic regression.

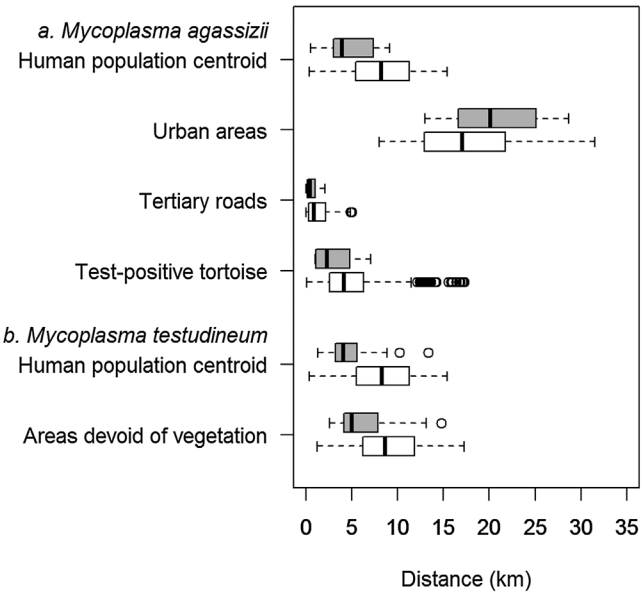

Figure 3. Boxplots comparing distances from test-positive and test-negative Agassiz's desert tortoises to nearest centroid of human-populated census blocks (abbreviated to human population centroid), urban area, tertiary road, test-positive tortoise, and area devoid of vegetation at a study area in the central Mojave Desert, California, 2005–2008. Distances are measured from test-positive (gray boxes) and test-negative (white boxes) tortoise locations for tortoises analyzed separately for a) *Mycoplasma agassizii* and b) *M. testudineum*. Boxplots display the median distance (center bars), first and third quartile (box edges), range of distances within a difference of 1.5 times the width of the box (whiskers), and extreme distances (points).

Single Variable Models and Regression Tree for *M. testudineum*

The distance to a human population centroid was also the best predictor for *M. testudineum* status in desert tortoises. The only other important predictor was distance to areas devoid of vegetation, which included playas (Coyote and West Cronese lakes) and agricultural areas (Table 3; Figs. 1 and 2). Numerous active and abandoned agricultural fields occur south-southeast of Coyote Lake and north of I-15, as well as throughout the Mojave River Valley. Positive or suspect disease status was negatively associated with both distance to a human population centroid and distance to areas devoid of vegetation, suggesting that tortoises located closer to human population centroids or to areas devoid of

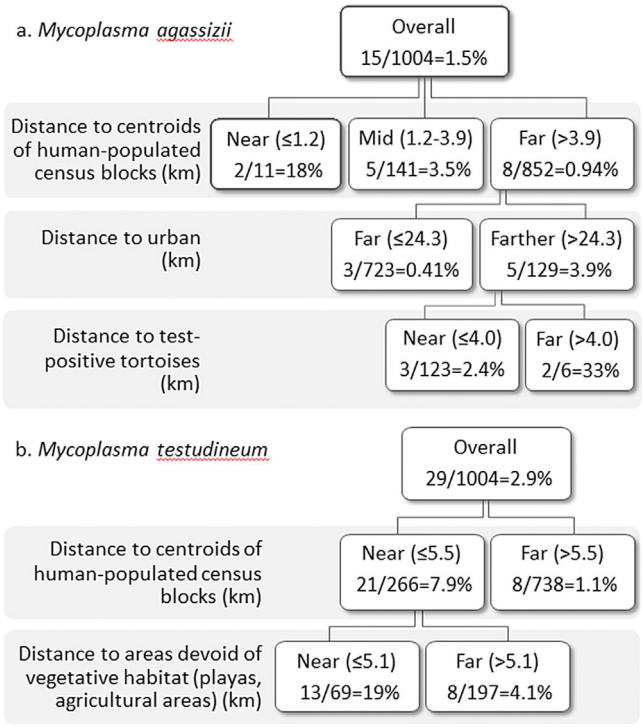

Figure 4. Variations in period prevalence (test-positive/*n* and % prevalence) of a) *Mycoplasma agassizi* and b) *M. testudineum* in Agassiz's desert tortoises in the central Mojave Desert of California between 2005 and 2008 using regression trees to partition covariate classes. Variations in *M. agassizi* period prevalence were best described by partitioning tortoises according to distance to centroids of human-populated census blocks, distance to urban, and distance to test-positive tortoises; and, in *M. testudineum*, according to distances to centroids of human-populated census blocks and to areas devoid of vegetation. Partitions were considered statistically significant at Bonferroni-adjusted $P < 0.05$, and rounded to 1 decimal place.

vegetation tended to experience higher prevalence of infection (Fig. 3). We found little collinearity between these variables (max. VIF = 1.1, $r = 0.62$).

In the regression tree with these 2 predictors, distance to a human population centroid was again the primary predictor partitioned because of a 7.9% (21/266) prevalence for test-positive tortoises ≤5.5 km from centroids of human-populated census blocks compared with 1.1% (8/738) for tortoises located farther away (Fig. 4). Among tortoises located within 5.5 km of a human population centroid, 19% (13/69) of tortoises closer to areas devoid of vegetation (≤5.1 km) were test-positive for *M. testudineum* compared with only 4.1% (8/197) of tortoises located >5.1 km from areas devoid of vegetative habitat.

DISCUSSION

Prevalence of *Mycoplasma* spp.

Prevalence of desert tortoises with antibody positive or suspect ELISA tests and or cultures for *M. agassizii* was low (1.49%) in this study. For most diagnostic assays, both NPV and PPV are affected when the true prevalence is either very low or very high (Smith 1995, Ott and Longnecker 2004). For the ELISA developed for *M. agassizii* in tortoises, the PPV drops below 90% only at a seroprevalence of <9% (Wendland et al. 2007). Therefore, the positive ELISA tests in our study population had the potential to be false positives. However, the isolation of *M. agassizii* and *M. testudineum* from the nares of tortoises provided confirmatory evidence that the pathogen was in fact present in the population and therefore the positive ELISA results were most likely true positives. Suspect results should as always be interpreted with caution. The 9 tortoises test-positive for *M. testudineum* exhibited lesions typical of URTD in the upper respiratory tract at necropsy, thereby providing additional support for true positive cases (Jacobson and Berry 2012).

Our findings of low prevalence for *M. agassizii* were similar to a study conducted between 1997 and 2003 with a smaller sample set at 21 sites north of our study area on the NTC, Ft. Irwin (Berry et al. 2006). In the earlier study, only 2 of 91 (2.2%) tortoises sampled were antibody positive for *M. agassizii* and 1 was culture positive. The prevalence in our study was low when compared to results of a multi-year health and disease research project conducted on small sets of tortoises in 3 different regions of the Mojave Desert between 1990 and 1995 (Christopher et al. 2003). At 1 site, the Desert Tortoise Research Natural Area in the western Mojave Desert, 45.7% of the tortoises tested were antibody positive for *M. agassizii* as measured by ELISA tests. This population experienced a decline of 90% associated with URTD in a brief period in the late 1980s and early 1990s (Brown et al. 1999). At the 2 other sites, Ivanpah Valley in the northeastern Mojave Desert and Goffs in the eastern Mojave Desert, tortoises were 12.5% and 32.1% seropositive and 39.4% and 28.1% culture positive, respectively (Christopher et al. 2003). The Goffs population also experienced a major decline in numbers. A similar range of seroprevalence was also observed in Nevada, where 15–23% of free-ranging tortoises sampled at Yucca Mountain had antibodies to *M. agassizii* (Lederle et al. 1997). The seroprevalence of *M. agassizii* in 11 populations of free-ranging gopher tortoises (*G. polyphemus*) over 5 years showed similar ranges of seroprevalences (<5–>60%; Wendland et al. 2010*b*); serological results were confirmed by positive culture, PCR, and presence of clinical signs.

No previously published data are available for prevalence of *M. testudineum* in wild desert tortoises. Tortoises were not tested for *M. testudineum* in the aforementioned studies, because this pathogen had yet to be identified and an ELISA test was not available (Brown et al. 1995, 2004).

Clinical Signs of Mycoplasmal URTD

Mycoplasmal URTD, like most respiratory mycoplasmal infections, is a chronic and often clinically silent infection. Even in experimentally infected tortoises that can be frequently and closely observed, individuals may vary in the suite and severity of clinical signs presented (Brown et al. 1994). Thus, the absence of clinical signs does not imply a healthy animal. The true measure of disease, the presence of lesions in the upper respiratory tract, is impossible to determine in live animal surveys. However, in studies of

necropsied tortoises (Jacobson et al. 1991, 1995; Brown et al. 1994), significant correlations existed among clinical signs of mycoplasmal URTD, presence of specific antibody to *M. agassizii*, and lesions in the upper respiratory tract demonstrated by histopathology and electron microscopy. For example, 92% of clinically ill tortoises had lesions of URTD, 50% had *M. agassizii* isolated from the nasal passages, and 100% reacted positively in the ELISA (Jacobson et al. 1995). Notably, 73% of tortoises with no overt clinical signs had lesions consistent with URTD; 50% were culture positive for *M. agassizii* at the time of necropsy, and 42% also were seropositive for antibodies against the pathogen. Importantly, tortoises without clinical signs of mycoplasmal URTD may have subclinical disease, lesions demonstrating substantive damage to the respiratory and olfactory tissues, and active colonization of the upper respiratory tract by the pathogen (Jacobson et al. 1995).

When tortoises are not subclinical, both nasal and ocular clinical signs can be indicative of tortoises that are antibody positive for *M. agassizii* and or have lesions in the respiratory tract (Jacobson et al. 1991, Schumacher et al. 1997, Christopher et al. 2003). Mucous nasal discharge is highly predictive for exposure to *M. agassizii* in *Gopherus* species, based on studies of the relationship between clinical signs of URTD and ELISA test results (Schumacher et al. 1997, Wendland et al. 2010*a*). Of 144 tortoises with a mucous nasal discharge, 93% tested seropositive using an ELISA test (Schumacher et al. 1997). Captive desert tortoises also have a positive association between positive ELISA test results and severity of clinical signs of URTD (Johnson et al. 2006). In our study, none of the 15 tortoises test-positive for *M. agassizii* had either a nasal discharge or moist, damp or wet naris or nares. However, 46.7% (7/15) of the tortoises showed clinical signs of a recent, dried nasal discharge (partially occluded or occluded naris or nares, dried exudate on beak or forelimbs). In the future, these more subtle signs of a potential or intermittent nasal discharge may be useful in assessing health.

Less is known about relationships between clinical signs, results of ELISA tests, and histopathology from necropsies for *M. testudineum* (Jacobson and Berry 2012). The 9 necropsied tortoises from this study all had antibody positive or suspect ELISA tests or positive cultures for *M. testudineum,* and lesions in the upper respiratory tract. Prior to necropsy, these tortoises were evaluated multiple times in the field and clinical signs of URTD were intermittent on those occasions. During the single evaluations used for data analyses here, 13.8% (4/29) of tortoises had a nasal discharge or moist, damp, or wet nares and an additional 31.0% (9/29) had clinical signs of a recent nasal discharge (partially occluded or occluded naris or nares, dried exudate on beak or forelimbs).

Distributions of *M. agassizii* and *M. testudineum*

Despite the visible differences in distribution of test-positive occurrences between the two pathogens in desert tortoises (Fig. 2), a direct statistical comparison did not reveal significant spatial differences. The lack of measurable difference could be due to several reasons. First, test-positive tortoises were few in number and often interspersed among test-negative tortoises. Second, large spatial overlaps occurred in areas where disease was not detected, as well as areas where few or no samples were collected. Third, given the low prevalence, we could not definitively confirm that such areas were disease-free even when all the tortoises we sampled were test-negative.

The distribution of the 2 *Mycoplasma* species on the landscape has implications for management of desert tortoises. The Fisher test did not indicate a strong statistical tendency for the 2 pathogens to co-occur; only 2 tortoises showed evidence for both *M. agassizii* and *M. testudineum*. Because the 2 species of *Mycoplasma* appear to have little overlap (<5%) and may be acting independently, it is important to test tortoises for both species of *Mycoplasma*, rather than only *M. agassizii,* for which more information is available. Testing for presence of infectious diseases in both resident tortoises and those scheduled to be translocated is critical, because the potential exists for each test-positive tortoise to infect other, nearby tortoises.

The primary variable predicting test-positive status for both *M. agassizii* and *M. testudineum* in desert tortoises was distance to a human population centroid, and it was likewise the first predictor partitioned in the regression trees. In all cases, prevalences of test-positive tortoises were negatively associated with distance to a human population centroid, which is suggestive of anthropogenic contributions to mycoplasmal URTD in desert tortoises. Overall, the human population within the study area was low, with census blocks having from 1 to 178 people (see Supplemental Information). Nearby were higher concentrations of people: in the southwest, the city of Barstow, and in the north, the Ft. Irwin cantonment. Additional unincorporated communities were adjacent or close to the southern boundary in the Mojave River Valley and each supported a population of <2,500 people (U.S. Census 2000 and local data; Fig. 1).

For *M. agassizii*, the regression tree indicated a secondary but positive association between prevalence and distance to urban edge applying to tortoises farthest from human population centroids. Our maps confirm a clump of test-positive tortoises located in the eastern end of the study area farthest from Barstow and the Ft. Irwin cantonment urban areas. Ill tortoises often exhibit abnormal behaviors (Berry and Christopher 2001), and long-distance travel outside of home ranges may be such a behavior. For example, McGuire et al. (2014) reported that home ranges of the related gopher tortoise (*G. polyphemus*) with severe URTD were significantly larger than home ranges of asymptomatic or mildly affected tortoises. They noted that tortoises with severe clinical disease may have a role in dispersing pathogens.

Other possible explanations are anthropogenic. One possibility is that test-positive tortoises, distant from human population centers, were among the thousands of captive tortoises released by state and other agency personnel as well as private citizens in the past (Murphy et al. 2007, Jacobson et al. 2014). Many unpublished accounts exist too, including

reports from caretakers who released their domestic pets to the wild regardless of disease status. The locations of these distant tortoises, seropositive for *M. agassizii,* are near the junction of an interstate highway exit road and a frequently used graded power line road (Figs. 1 and 2). A less likely explanation is that research scientists served as fomites and spread the disease. The locations of the distant test-positive tortoises were near research study areas used by several scientists between the early 1990s and 2008. Although protocols to prevent spread of infectious diseases were available (Berry and Christopher 2001), the protocols may not have been used or were ineffective. We recognize that factors potentially unique to this area may have contributed to the disease status of these tortoises, and large distance to an urban area may simply be the best surrogate in our dataset for describing this location.

Distance to other test-positive tortoises was another tertiary predictor for *M. agassizii* with a less clear relationship. The logistic regression indicated that tortoises were more likely to be test-positive when they were closer to other test-positive tortoises. The regression tree did not reveal a similar pattern, perhaps because this influence is masked by a similar influence due to distance to a human population centroid. Instead, an opposite relationship emerged. For tortoises that were far from humans, the test-positive tortoises tended to be farther from other test-positive tortoises. This predictor, unlike other predictors based on spatial fixtures and comprehensively mapped, was subject to sampling biases. The test-positive tortoises represented only a subset of the entire population. Thus, some tortoises in our sample could have been closer to a test-positive neighbor than we measured. Also distances between tortoises were not mutually independent, which may potentially exaggerate the significance of the effect. The most likely explanation is that a separate cohort of diseased tortoises existed, originating from either infected resident tortoises or previously owned and released captive tortoises. This cohort characteristically may be remote from urban areas and towns, in areas with low human population densities.

In contrast to *M. agassizii*, for *M. testudineum*, the only other important predictor was distance to playas and agricultural fields, which, like distance to a human population centroid, was also negatively associated with prevalence. Playas are sinks and potential concentration areas for toxicants transported by wind and water from mines, roads, and other disturbed areas (Chaffee and Berry 2006; Kim et al. 2012, 2014). Agricultural fields are treated with fertilizers and chemicals. Both playas and agricultural areas are sources of fluvial and windborne dust and deposits that can contaminate tortoise food plants and habitat on adjacent lands, thereby affecting tortoise health (Seltzer and Berry 2005, Chaffee and Berry 2006). We did not find evidence of a relationship between either of the 2 mycoplasmal diseases and the remaining covariates: slope, aspect, elevation, vegetation, sex, and carapace length.

Distance from human population centroids may be a reasonable predictive tool for explaining mycoplasmal status of wild desert tortoises. The relationship that we report here for *M. agassizii* was similar to a finding described for *M. agassizii* for tortoises within Ft. Irwin boundaries (Berry et al. 2006). In this latter study, rates of tortoises that were antibody positive or culture positive were negatively correlated with distances from Ft. Irwin offices, the cantonment, and paved roads. In our case, as with the previous study (Berry et al. 2006), the epidemiology of mycoplasmosis may be in early stages. The proximity to humans may also be a source of stress to wild tortoises, thus increasing vulnerability to disease. Stressors can be in the form of handling tortoises (Berry et al. 2002); degraded and fragmented habitat; encounters with and injuries from domestic dogs, other pets, and subsidized predators (Esque et al. 2010); injuries from vehicles; and trash and other discarded waste materials (Donoghue 2006). Other diseases, such as herpesvirus, which occur in both captive and wild tortoises, may be involved too (Johnson et al. 2006, Jacobson et al. 2012) and may further influence tortoise health.

Human-population centroids in turn may be closely associated with escaped or released captive desert tortoises and other species of turtles and tortoises. Desert tortoises have been and are popular as domestic pets. In 1973, 17 years before the species was listed as threatened under the California Endangered Species Act (1989) and federal Endangered Species Act (USFWS 1990), the State of California passed regulations to prohibit take of wild tortoises (State of California, Title 14. 674). People who already possessed desert tortoises were allowed to keep them (State of California, Title 14. 674). Thousands of captive tortoises exist in southern California, and this population has continued to grow through unauthorized breeding and illegal take. For example, between 1998 and 2012 an estimated 500–900 tortoises were turned over annually to the California Turtle and Tortoise Club for adoption (annual summaries of adoptions for 1998–2012 available at http://www.tortoise.org/). At the NTC, biologists have reported escaped captive desert tortoises and other species of tortoises (e.g., *Testudo horsfieldii*; M. Massar and L. Aker, U.S. Army, Ft. Irwin National Training Center; personal communication). Biologists also have translocated wild tortoises within base boundaries, if found in harm's way (M. Massar, personal communication).

Escaped and intentionally released captive tortoises are sources of mycoplasmal URTD. Many captives have clinical signs of URTD and are antibody positive for *M. agassizii* (Johnson et al. 2006). In a survey of desert tortoises in the Greater Barstow Area, 21.3 km southwest of the study area, 82.7% of captive tortoises ($n = 179$) were antibody positive for *M. agassizii*. No tests were undertaken for *M. testudineum* in the Johnson et al. (2006) study. The prevalence of tortoises antibody positive for *M. agassizii* was also high on tracts of land within and adjacent to the city of Las Vegas in Las Vegas Valley in 1990; 50% of 144 tortoises tested were antibody positive (Schumacher et al. 1997). Jones (2008) observed a similar pattern in the closely related *G. morafkai* (see Murphy et al. 2011 for recent taxonomic revision) in Arizona: the prevalence of antibody-positive *G. morafkai* was

higher in captive tortoises in suburban areas than at remote sites.

Within and adjacent to the study area are potential sources of infected captive tortoises: the 2 urban areas, unincorporated communities, and numerous households (Fig. 1). More housing units or households are present in the western portion of the study area in close proximity to the Ft. Irwin Road and urban areas than in the east. In a study of captive desert tortoises in nearby desert towns, 98% of tortoises were in households with multiple tortoises, and 12% of these households had other species of turtles and tortoises (Edwards and Berry 2013). Captive tortoises with URTD have potential to escape or be deliberately released and thus contribute to spillover of infection from domestic to wild populations.

Although we have emphasized proximity to humans as a risk to exposure and to likelihood of finding test-positive tortoises, other stressors such as drought and toxicants may increase vulnerability to disease. Tortoises have physiological and behavioral adaptations for coping with drought (Henen et al. 1998, Christopher et al. 1999), which is a common occurrence in the Mojave Desert (Hereford et al. 2006). Climate warming with the prospect of increasing severity and frequency of droughts (Seager et al. 2007, Garfin et al. 2014) may exceed existing physiological tolerances of tortoises and affect health. For example, Christopher et al. (2003) reported that tortoises entering hibernation in a drought year may be physiologically compromised. Tortoises emerging from hibernation in years following a period of drought were more likely to have clinical signs of URTD; additionally, tortoises with oral lesions were significantly more likely to be dehydrated and to have positive nasal cultures for *M. agassizii* (Christopher et al. 1995). Likewise, exposure to toxicants (e.g., mercury, arsenic, lead) may increase susceptibility to or severity of disease (Jacobson et al. 1991, Seltzer and Berry 2005). The source of such toxicants can be windborne dust and deposits in ephemeral stream channels from mining, roads, recreational vehicle use, and other human uses (Seltzer and Berry 2005, Chaffee and Berry 2006, Kim et al. 2014).

In the end, anthropogenic factors appear to be important drivers in the 2 *Mycoplasma* spp., as have been described for some other emerging infectious diseases (Daszak et al. 2001). In the case of the desert tortoise, potential explanations for the origin of mycoplasmosis in wild populations may lie in spillover from infected captive tortoise populations rather than with the endemic pathogen hypothesis (Rachowicz et al. 2005). With the projected expansion of human populations within the geographic range of Agassiz's desert tortoise (Hunter et al. 2003), the potential for spillover of *Mycoplasma* spp. and other infectious diseases to wild tortoise populations will grow, thus hampering recovery efforts for this threatened species (USFWS 2011).

MANAGEMENT IMPLICATIONS

Gopherus agassizii and other species of turtles and tortoises face many anthropogenic threats that have contributed to extinctions of chelonians in the past (Rhodin et al. 2011). One threat, URTD caused by *M. agassizii*, has been well studied in desert tortoises. Mycoplasmal URTD occurs in the other 4 species of gopher tortoises, *G. belandieri, G. flavomarginatus, G. morafkai,* and *G. polyphemus* and these species are also protected with threatened or endangered status in all or parts of their geographic ranges (Jones 2008, Brown et al. 2001, Wendland et al. 2006, Truett and Phillips 2009). Mycoplasmal URTD also has been reported in several other genera and species of captive tortoises from Europe, Asia, and Africa (Wendland et al., 2006). Thus, our findings have implications for management of other species of tortoises worldwide, especially where captives are kept as pets or in colonies with other species of tortoises, are used in augmentation or recovery programs, and have counterparts in nearby wild populations. First, we reported that the distribution of tortoises with *M. agassizii* and *M. testudineum* on the landscape has implications for management because the 2 pathogens were infrequently detected in the same tortoises. Protocols for testing for infectious diseases should include species of *Mycoplasma*, not just the better known *M. agassizii*, as well as herpesviruses (Jacobson et al. 2012). Second, our findings support earlier work that clinical signs of mycoplasmal URTD may be absent or subtle, and that laboratory tests are an essential part of determining status of diseases in desert tortoises and potentially other chelonians. Third, the association between desert tortoises that are test-positive for *M. agassizii* or *M. testudineum* and proximity to human households is of sufficient importance to develop management strategies to reduce disease transmission, such as signing and fencing the boundaries of critical habitats in close proximity to human households and urban areas. The recovery team for desert tortoises previously recommended such strategies for dealing with the numerous human-related impacts occurring at the urban-desert edge (USFWS 1994). When wild tortoises are translocated, the proximity to human households and settlements should be a consideration. Because escape or intentional release of captive tortoises with mycoplasmal URTD may be a source of disease in wild populations, management of captive populations of chelonian species also is essential.

ACKNOWLEDGMENTS

We thank the field team, including C. Furman, L. Acosta, T. Bailey, S. Boisvert, J. Boswell, M. A. Hasskamp, W. Hasskamp, S. Hanner, E. Holle, D. Hinderle, J. Hillman, C. Keaton, K. Kermoian, P. Kermoian, K. Lucas, R. McGuire, T. Ose, K. Palmer, D. Silva, T. Shields, R. Woodard, and A. P. Woodman. P. Woodman, A. Walde, K. Drake, T. Esque, and K. Nussear coordinated fieldwork with additional research teams. We thank the research staff of the University of Florida Mycoplasma Diagnostic Laboratory for technical assistance; R. Lugo, L. Scott, and L. Beale for advice on ArcGIS and spatial analysis; K. Phillips, B. Halstead, S. Jones, and R. Averill-Murray and two anonymous reviewers for constructive reviews; and to W. Quillman and C. Everly for advice. Both the NTC and the U.S. Geological Survey provided financial support. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. government.

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Associate Editor: Paul Cross.

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